I and mannosidase II localized in the ER or Golgi apparatus of the host cell, the method comprising the step of introducing into the host cell a nucleic acid encoding a GlcNAc transferase II enzyme comprising:

(a) a catalytic domain having GlcNAc transferase II activity selected to have a pH optimum within 1.4 pH units of the average pH optimum of glycosylation-related enzymes in a subcellular location where the domain is targeted; and

(b) a cellular targeting signal peptide not normally associated with the catalytic domain selected to target the GlcNAc transferase II enzyme to the ER or Golgi apparatus of the host cell;

whereby, upon passage of the recombinant glycoprotein through the ER or Golgi apparatus of the host cell, a recombinant glycoprotein comprising a $GlcNAc_2Man_3GlcNAc_2$ glycoform is produced.

REMARKS

Applicant and his representatives first wish to express their gratitude to the Examiner for the interview of February 10, 2005 ("the Interview") and for the Examiner's continued willingness to discuss claim amendments to place the application in condition for allowance. The claim amendments submitted herewith are those that were discussed in detail with the Examiner during that interview, as described in more detail below.

I. Claim Amendments

Claims 35, 39, 40, 42-50, 52-54 and 57-73 are currently pending. Claims 1-34, 36-38, 41, 51, 55 and 56 have been canceled previously. With this response, claims 42, 43 and 54 have been

canceled, claims 35, 40, 44-48, 50, 52, 58, 59, 61-64 and 68-73 have been amended, claims 74-78 are non-entered, and claims 79-80 have been added to define more clearly what applicant considers to be his invention. Each of these amendments is supported by the specification as originally filed and none adds new matter. The amendments are addressed below in the context of addressing the Examiner's outstanding rejections.

A. 35 U.S.C. § 112 Claim Rejections – Written Description

Claims 35, 39, 40, 42-50, 52-54 and 57-69 and 71-73 stand rejected under 35 U.S.C. § 112, first paragraph, for lack of adequate written support in the application as originally filed. Claims 42, 43 and 54 have been canceled, rendering this rejection moot as to those claims. The Examiner acknowledges that the written description requirement has been met for a "lower eukaryotic host cell that does not display *alpha-1,6 mannosyltransferase activity* with respect to the N-glycan on a glycoprotein" (Action, page 4). But the Examiner points out that the rejected claims are not limited to the alpha-1,6 mannosyltransferase, as they recite a "lower eukaryotic host cell that does not display *a 1,6 mannosyltransferase activity* with respect to the N-glycan on a glycoprotein". And, according to the Examiner, "[t]he specification does not disclose a lower eukaryotic host cell lacking any activity of other 1,6 mannosyltransferase with respect to N-glycan of the glycoprotein" (*Id.*). Applicant has amended claim 35 to limit the recited 1,6-mannosyltransferase activity to an "alpha-1,6 mannosyltransferase" activity, thus obviating this rejection. Applicant notes that pending independent claim 71 (and hence dependent claims 72 and 73) already recites the "alpha" limitation and hence has not been further amended. Accordingly, applicant requests that the Examiner withdraw the 112 rejections based on this term.

Claims 35, 39, 40, 42-50, 52-54 and 57-69 and 71-73 also stand rejected under 35 U.S.C. § 112, first paragraph, for lack of adequate written support in the application with respect to "a mannosidase enzyme for the production of a $Man_5GlcNAc_2$ carbohydrate structure." The Examiner states that "[a]lthough the specification provides a number of alpha 1,2 mannosidase from different species, it still does not satisfy the written description requirement for any hybrid mannosidase enzymes as claimed." (Action, page 5). Accordingly, applicant has amended claims 35 and 71 (and hence claims that depend therefrom) to specify that the mannosidase enzyme introduced into the host cell has alpha 1,2 mannosidase activity.

Specifically, each of amended claims 35 and 71 recites: "the enzyme comprising: (a) a catalytic domain having alpha-1,2 mannosidase activity." In addition, for clarity, applicant has deleted the term "hybrid" from the claims, as the term is redundant with the recitation that the claimed enzyme comprise (a) a "catalytic domain" and (b) a "cellular targeting signal peptide not normally associated with the catalytic domain." New claims 79 and 80 are also supported by the application as originally filed and meet the § 112 written description requirement as well, as discussed with the Examiner at the Interview.

Based on the above amendments, applicant respectfully requests that the Examiner withdraw the outstanding claim rejections based on lack of adequate written description.

B. 35 U.S.C. § 112 Claim Rejections – Enablement

Claims 35, 39, 40, 42-50, 52-54 and 57-73 stand rejected under 35 U.S.C. § 112, first paragraph, for lack of enablement. Claims 42, 43 and 54 have been canceled, rendering this rejection moot as to those claims. In particular, the Examiner contends that the specification "does

not reasonably provide enablement for a method for producing *any* type of humanized protein with *any* type of complex glycoprotein structure (even when the intermediate $Man_5GlcNAc_2$ structure is produced.) (Action, page 6; emphasis added.) The Examiner also contends that the specification does not enable humanized protein production "without introducing [an] exogenous construct encoding such protein." *Id.* The Examiner affirms, however, that the original specification *does* enable methods for producing glycoproteins in lower eukaryotic host cells that have been transfected or co-transfected with nucleic acid constructs that encode the protein of interest to be glycosylated, and one or more of: alpha-1,2 mannosidases I and II, N-acetylglucosaminyl transferases I (GnTI) and II (GnTII), and a UDP-acetylglucosamine transporter. As detailed below, although applicant traverses these enablement rejections, applicant's claim amendments nonetheless obviate the Examiner's rejections.

Claims 35, 59, 70 and 71 have been amended to delete the term "humanized" and replace it with "recombinant." As amended, claims 35, 70 and 71 thus recite "[a] method for producing a recombinant glycoprotein" and dependent claims 54 and 59 refer to "production of the glycoprotein." New claims 79-80 also recite this phrase. Support for this amendment is found, e.g., at page 11, lines 28-30 (see also, e.g., page 8, line 26; page 9, lines 7 and 11; and page 12, line 6) of the original specification.

Claims 35, 70 and 71 have also been amended to recite that the recombinant glycoprotein produced by the method comprises particular N-glycoforms. Claim 35, as amended, recites that the recombinant glycoprotein produced according to the method comprises a $Man_5GlcNAc_2$ glycoform, and that "upon passage of the recombinant glycoprotein through the ER or Golgi apparatus of the host cell, in excess of 30 mole % of the N-glycan structures attached thereto have a $Man_5GlcNAc_2$

glycoform that can serve as a substrate for GlcNAc transferase I *in vivo*." And, as amended, claims 70 and 71 recite that the recombinant glycoprotein produced according to the method comprises N-glycan structures comprising $GlcNAcMan_5GlcNAc_2$. Similarly, new claims 79 and 80 recite methods for producing recombinant glycoproteins with an N-glycan comprising a $GlcNAcMan_3GlcNAc_2$ and a $GlcNAc_2Man_3GlcNAc_2$ glycoform structure, respectively.

Claims 35, 40, 62, 70 and 71 have also been amended to recite the step of introducing into the host cell one or more "nucleic acids encoding" the one or more enzymes recited in the claims. New claims 79 and 80 also recite this technical feature. Similarly, claims 50, 63, 64 and 70 have been amended to recite (and new claims 79 and 80 recite) that the lower eukaryotic host cell is/has been "genetically modified" to express the one or more recited enzymes, to clarify that it is not a protein having enzymatic activity that has been introduced into the host cell.

Addition of the term "recombinant" and the requirement that certain activities be expressed from nucleic acids overcomes the Examiner's rejection based on lack of enablement to produce "*any* type of humanized protein," as the amended claims are now limited to recombinantly expressed proteins. Addition of the term "recombinant" and the limitation to expression from nucleic acids also overcomes the Examiner's rejection based on her assertion (which is traversed[1]) that the specification fails to enable a method of producing a humanized protein in a lower eukaryotic host cell without introducing an exogenous construct encoding such a protein.

[1] The claimed methods may be used to produce recombinant glycoproteins in lower eukaryotic host cells that have been *previously* engineered to express a recombinant protein. And, in fact, the invention is not limited to producing a protein-of-interest from an exogenous DNA introduced into the host cell. Host cell endogenous proteins will also enter the host's engineered secretory pathway and become glycosylated with human-like N-glycan structures.

In addition, claims 35, 70 and 71 have been amended to (and new claims 79 and 80) recite that it is "upon passage of the recombinant glycoprotein through the ER or Golgi apparatus of the host cell" that the desired modified N-glycan structure is produced.

Deletion of the term "humanized," insertion of the term "recombinant," recitation of particular N-glycan structures and a requirement for expression from nucleic acids or for use of "genetically modified" host cells in the amended claims overcomes the Examiner's rejections based on lack of enablement to produce "any type of humanized protein with *any* type of complex glycoprotein structure (even when the intermediate $Man_5GlcNAc_2$ structure is produced.)" (Action, page 6.) The claims as amended herein no longer encompass a method for producing *any* type of humanized protein with *any* type of complex glycoprotein structure. Applicant thus respectfully requests that the Examiner withdraw her rejections on this basis.

The Examiner has also rejected claims 71-73 for lack of enablement because "the specification does not teach how to convert long mannose chain N-glycan structure to humanized glycoprotein without the trimming by mannosidase to first produce the intermediate $Man_5GlcNAc_2$ structure." (Action, page 6.) Claim 71 has thus been amended to recite that the method comprises "the step or steps of introducing into the host cell at least two enzymes," one having alpha-1,2 mannosidase activity and the other having GlcNAc transferase I activity. Support for this amendment is found throughout the original specification; see, e.g., page 14, lines 11-23, and page 16, lines 6-21; page 20, line 8 – page 25, line 20; and Example 2 at page 33. Applicant thus respectfully requests that the Examiner withdraw these rejections of claims 71-73.

New claims 79-80 recite a method for producing recombinant glycoproteins in a lower eukaryotic host cell that has been genetically modified to produce N-glycan structures having an excess of 30 mole % of a $Man_5GlcNAc_2$ glycoform converted *in vivo* to $GlcNAcMan_5GlcNAc_2$ (claim 79) or $GlcNAcMan_3GlcNAc_2$ (claim 80) by enzymes localized in the ER or Golgi apparatus of the host cell.[2] Each claim recites the step of introducing a nucleic acid encoding an additional enzyme selected for optimal activity in the ER or Golgi apparatus of the particular host cell being used, the additional enzyme (mannosidase II in claim 79 and GnTII in claim 80) comprising (a) a catalytic domain selected to have a pH optimum within 1.4 pH units of the average pH optimum of glycosylation-related enzymes in a subcellular location where the domain is targeted; and "(b) a cellular targeting signal peptide not normally associated with the catalytic domain selected to target the catalytic domain to said ER or Golgi apparatus." Original support for new claims 79-80 is found throughout the original specification; see, e.g., page 14, lines 14-21; page 16, lines 6-21 (and see page 2, lines 15-26 for "complex N-glycans"); page 25, line 15 to page 26, line 6; page 27, lines 5-18 and Examples 2-7 at pages 33-38; see also original claims 12-16.

Post-filing date evidence of enablement

Applicant acknowledges with appreciation the Examiner's recognition that the claimed method is enabled to the extent of what is demonstrated in Choi et al. and Hamilton et al. (Action,

[2] Applicant submits new claims 79-80 to expedite allowance of claims and without prejudice for submitting claims of broader scope in one or more related applications claiming priority from the instant application to pursue similar claims that are not limited to using a host cell that is "genetically modified" to produce the recited N-glycan structures that act as substrates for the nucleic acid encoded enzyme introduced by the recited method. The original application states clearly that such a host cell may either be selected from nature or created by genetic modification (page 16, lines 6-26).

page 8.) New claims 79-80 are also supported by the application as filed, and encompass methods of the invention demonstrated by Choi et al. and Hamilton et al. to produce recombinant glycoproteins with N-glycans comprising $GlcNAcMan_3GlcNAc_2$ (claim 79) and $GlcNAc_2Man_3GlcNAc_2$ (claim 80) glycoforms.

Based on the above, applicant respectfully requests that the Examiner withdraw the outstanding enablement rejections.

C. 35 U.S.C. § 112 Claim Rejections – Indefiniteness

Claims 58 and 61 remain rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for reciting the term "derived." Applicant has deleted this term from the claims, thus obviating the rejections.

Claims 35, 39, 40, 42-50, 52-54, and 57-70 stand rejected under 35 U.S.C. § 112, second paragraph, for being unclear and incomplete for failing to bridge the gap between steps that produce a $Man_5GlcNAc_2$ glycoform and steps that produce a desired "humanized" glycoprotein. Claims 42, 43 and 54 have been canceled, rendering this rejection moot as to those claims.

Applicant traverses the Examiner's characterization that a "gap" exists between the $Man_5GlcNAc_2$ "intermediate" and "humanized" glycoforms, which is contrary to the teaching of the application as a whole (the application teaches that the $Man_5GlcNAc_2$ "intermediate" is "humanized" compared to N-glycans made in a wild-type lower eukaryotic host cell.) Nonetheless, as amended, those claims no longer characterize the glycoprotein as being "humanized." Deletion of the term "humanized" from claims 35, 59 and 70 (and deletion of "human-like" from claim 71) thus obviates the Examiner's rejection with respect to the use of this term. As amended, the claims

clearly recite that glycoproteins comprising desired glycoform structures have been produced in host cells that have expressed glycoproteins comprising a $Man_5GlcNAc_2$ N-glycan structure. In certain embodiments, (i.e., if the host cell displays additional glycosylation enzymatic activities as taught in the application), the $Man_5GlcNAc_2$ N-glycan structure may be further processed within the host cell (e.g., to $GlcNAcMan_5GlcNAc_2$, $GlcNAcMan_3GlcNAc_2$ and/or $GlcNAc_2Man_3GlcNAc_2$.)

D. Amendments For Further Clarity

Applicant has amended independent claims 35, 70 and 71 to recite more clearly what he considers to be the invention. In particular, as amended, claims 35, 70 and 71 now specify that the introduced glycosylation enzyme comprises a specified catalytic domain and a "cellular targeting signal peptide not normally associated with the catalytic domain selected to target the catalytic domain to the ER or Golgi apparatus of the host cell." Original support for these amendments may be found throughout the specification, e.g., at page 12, lines 13-19; page 14, lines 3-6 and 11-13; page 21, lines 3-16; page 28, lines 1 – page 30, line 3; and page 31, lines 7-12; original claim 1.

Claim 39 has been amended to clarify that the mannosidase "enzyme" is targeted within the host cell.

Claims 44 and 45 have been amended for clarity and consistency of claim language to recite that the recombinant glycoprotein comprising the N-glycan is further modified to comprise one or more recited sugars.

Claim 46 has been amended to additionally depend from independent claims 70, 79 or 80.

Claim 47 has been amended to clarify that the host cell "further lacks" the recited enzymatic activities.

Claim 48 has been amended to specify that the host cell does not express an "enzyme activity with respect to the N-glycan on a glycoprotein," for clarity and consistency with the language of claim 35, from which it depends.

Claim 52 has been amended to specify that the recombinant glycoprotein is isolated "subsequent to passage of the recombinant glycoprotein through the ER or Golgi apparatus of the host cell."

Claim 58 has been amended to replace the Greek letter symbol alpha with the term "alpha" for consistency of claim language.

Claim 59 has been amended to clarify that it is at least one of the "additional enzymes" that is localized "in the host" by forming the recited fusion protein and to change the dependency to claim 40 from claim 54, now canceled.

Claims 68 and 69 have been amended to delete their dependencies from claim 54, now canceled.

Claims 72 and 73 have been amended to add additional claim dependencies. Claim 73, part (a) has also been amended to delete "MALDI-TOF-MS" which is merely an example of the recited "mass spectroscopy."

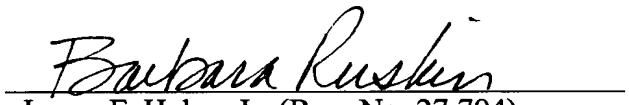
Each of the above amendments is supported by the application as originally filed and none adds new matter.

Based on the above amendments, applicant respectfully requests that the amended claims be favorably considered and allowed.

II. Conclusion

Entry of this Amendment and allowance of the claims as submitted herewith is respectfully requested.

Respectfully submitted,



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